



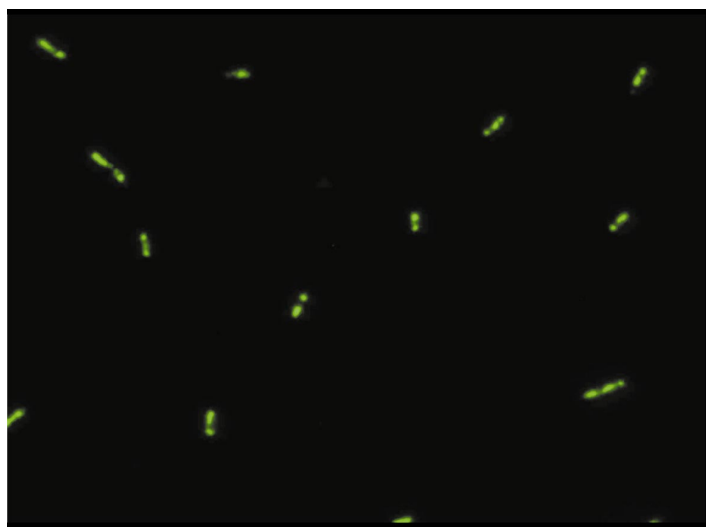
The Effect of Microgravity on the Smallest Space Travelers

Bacterial Physiology and Virulence on Earth and in Microgravity

Since the first human flights outside of Earth's gravity, crew health and well-being have been major concerns. Exposure to microgravity during spaceflight is known to affect the human immune response, possibly making the crew members more vulnerable to infectious disease. In addition, biological experiments previously flown in space have shown that bacteria grow faster in microgravity than they do on Earth.

The ability of certain antibiotics to control bacterial infections may also differ greatly in microgravity. It is therefore critical to understand how spaceflight and microgravity affect bacterial virulence, which is their ability to cause disease. By utilizing spaceflight hardware provided by the European Space Agency (ESA), Dr. Barry Pyle and his team at Montana State University, Bozeman, will be performing an experiment to study the effects of microgravity on the virulence of a common soil and water bacterium, *Pseudomonas aeruginosa*. Importantly, these bacteria have been detected in the water supplies of previous Space Shuttle flights. The experiment will examine the effects of microgravity exposure on bacterial growth and on the bacterium's ability to form a toxin called *Exotoxin A*.

Another goal is to evaluate the effects of microgravity on the physiology of the bacteria by analyzing their ability to respire (produce energy), by studying the condition of the plasma membrane surrounding the cell, and by determining if specific enzymes remain active. Proteins produced by the bacteria will also be assayed to see if the normal functions of the bacteria are affected. In the context of human life support in spaceflight, the results of this experiment will



Bacterial respiration in response to microgravity is measured by providing cells with an indicator of respiratory enzyme activity, r-iodonitrotetrazolium violet (INT). Respiring cells take up INT and convert it to an insoluble form. Upon return to Earth, the cells are visualized via staining with the fluorescent molecule SYBR Green, and cells that were respiring on orbit (fluorescent cells containing black spots) are counted.

offer guidance in providing the highest possible water quality for the Shuttle in order to limit the risk of infection to human occupants and to minimize water system and spacecraft deterioration.

Earth Benefits and Applications

This experiment will provide valuable insight to the field of microbiology regarding the growth, physiology, and virulence of the common soil and water bacterium, *P. aeruginosa*, that is also able to cause disease in humans.

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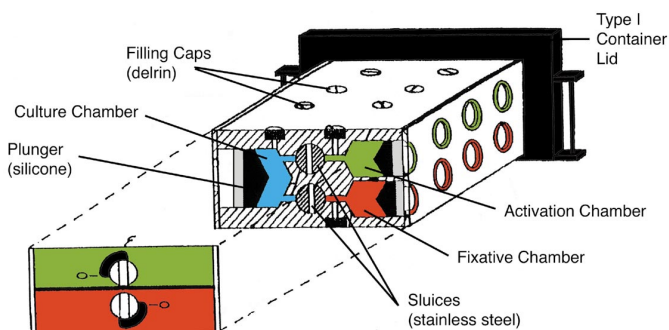
Background Information

Science

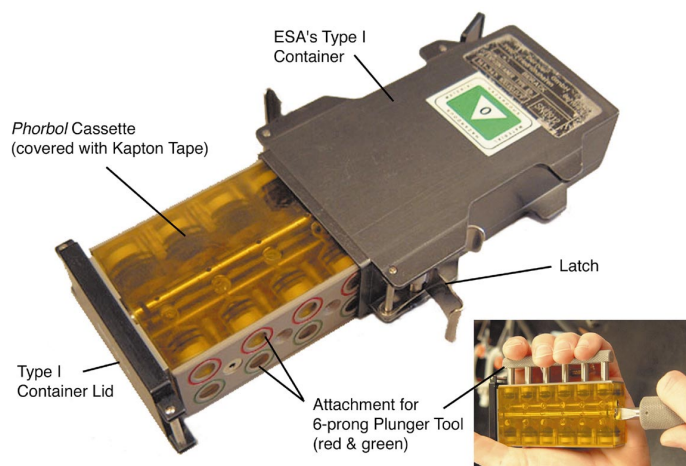
Pathogenic species such as *P. aeruginosa* cause disease by producing toxins that affect the physiological functions of the host cells. Before launch, a strain of *P. aeruginosa* that produces Exotoxin A will be cultured in the laboratory under conditions that repress toxin production. Samples will be loaded into the cassettes and kept at +5 °C until they are activated on orbit to stimulate toxin production. The cassettes will be incubated for 24±1 hours at +37 °C, exposed to various treatments, and refrigerated at +5 °C until landing. Upon return to Earth, assays for exotoxin production, cell toxicity, proteins, and bacterial physiology will be performed.

Hardware

This experiment will be performed using ESA's Biopack spaceflight hardware which provides an incubator with centrifuges and a built-in cooler. On board the Space Shuttle, samples can be exposed to gravity levels ranging from microgravity to twice Earth's gravity. Biopack is designed to accommodate small biological samples, e.g. bacterial cultures, mammalian cell, tissue cultures and small plants or



The experiment cassettes developed for the Biopack hardware contain the bacteria samples in culture chambers that will be activated in flight. Each Phorbol cassette contains six culture chambers (left side of cassette), six activation chambers (upper chambers, right side) and six fixative chambers (lower chambers). Pre-flight, cultures and solutions are loaded into their respective chambers. At the time of activation, the green/activation sluice is turned to the "open" position and the contents of the activation chambers is injected into the culture chambers using a plunger tool. The sluice is then closed and the cassettes are incubated. At the time of termination, a similar procedure is followed (red/termination sluice).



Science Discipline Supported

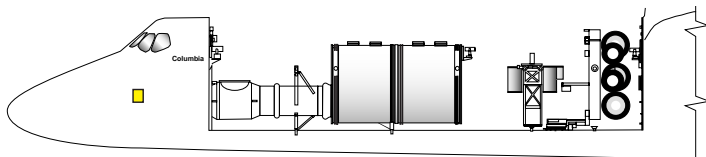
This experiment supports NASA's priorities for research aimed at understanding and alleviating problems that may limit astronauts' ability to survive and/or function during prolonged spaceflight, and will provide the field of microbiology valuable insight into the growth, physiology, and virulence of the common soil and water bacterium, *P. aeruginosa*.

insects. Dr. Pyle's experiment use the +37 °C incubator, while pre- and post-incubation refrigeration will be provided by a +5 °C Passive Thermal Conditioning Unit (PTCU).

The cultures will be housed in experiment cassettes that are contained in sealed containers. The experiment will utilize a total of 16 of these devices. Eight containers will comprise the flight set and eight containers will comprise the ground control set. On flight day 8, the astronaut crew will activate the cultures by pushing six activator plungers to transfer the bacteria into the growth medium. The containers will then be placed in the Biopack +37 °C incubator for 24 hours. For the flight experiment, four containers will be incubated in a stationary holder to expose the bacteria to the effects of microgravity, and four containers will be centrifuged for the on-orbit 1g control. Following incubation the crew will return the cassettes to refrigeration for the duration of the flight.

Previous Results

Preliminary data analysis from the NASA/ESA Biorack program (STS-81, Shuttle *Atlantis*, January 1997) suggests that the numbers of attached cells in biofilms grown in microgravity were several times greater than their Earth-based counterparts. The STS-107 experiment proposes to utilize this research opportunity to obtain further data on the effects of microgravity and spaceflight on the growth, physiology and virulence of waterborne bacteria. The results from this experiment will further our understanding of bacterial behavior in spaceflight and on Earth.



Approximate location of this payload aboard STS-107.

Picture credits. Pyle (page 1), Ames Research Center (page 2)

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